McSwiggen et al.

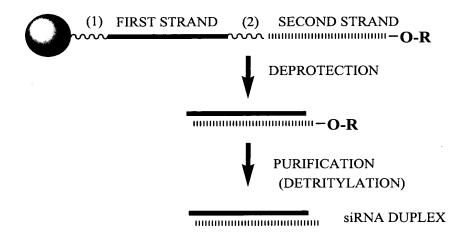
RNA Interference Mediated Treatment of Alzheimer's

Disease Using Short Interfering Nucleic Acid (siNA)

Serial No. TBA; MBHB02-728-B (400/120)

Sheet 1 of 13

# Figure 1



= SOLID SUPPORT

R = TERMINAL PROTECTING GROUP FOR EXAMPLE: DIMETHOXYTRITYL (DMT)

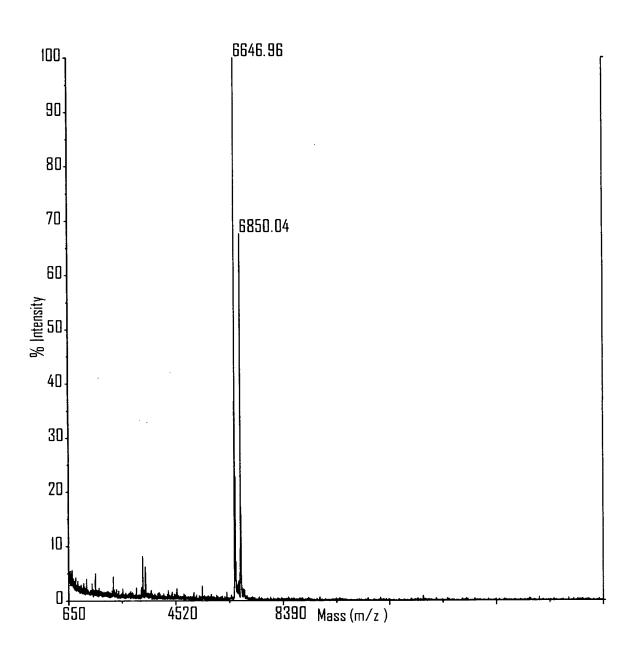
(1) = CLEAVABLE LINKER
(FOR EXAMPLE: NUCLEOTIDE SUCCINATE OR
(2) INVERTED DEOXYABASIC SUCCINATE)

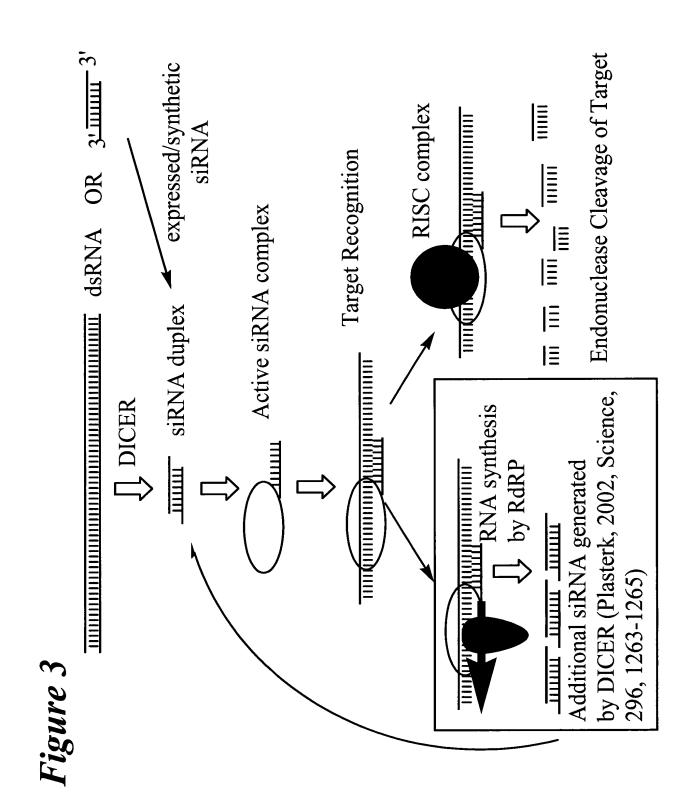
= CLEAVABLE LINKER
(FOR EXAMPLE: NUCLEOTIDE SUCCINATE OR INVERTED DEOXYABASIC SUCCINATE)

INVERTED DEOXYABASIC SUCCINATE LINKAGE

GLYCERYL SUCCINATE LINKAGE

# Figure 2





McSwiggen et al.
RNA Interference Mediated Treatment of Alzheimer's Disease Using Short Interfering Nucleic Acid (siNA) Serial No. TBA; MBHB02-728-B (400/120) Sheet 4 of 13

# Figure 1

	rigure 4
A	SENSE STRAND (SEQ ID NO 691) ALL POSITIONS RIBONUCLEOTIDE EXCEPT POSITIONS (N N)
	5'- B-NNNNNNNNNNNNNNNNNNNNNNN (NN)-B -3'
	$\int 3'$ - L- $(N_sN)$ NNNNNNNNNNNNNNNNNNN -5'
	ANTISENSE STRAND (SEQ ID NO 692) ALL POSITIONS RIBONUCLEOTIDE EXCEPT POSITIONS (N N)
В	SENSE STRAND (SEQ ID NO 693) ALL PYRIMIDINES = 2'-FLUORO AND ALL PURINES = 2'-OM EXCEPT POSITIONS (N N)
	5'- NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
	3'- L-(N <sub>s</sub> N) NNNNNNNNNNNNNNNNNNNNNN -5'
	ANTISENSE STRAND (SEQ ID NO 694) ALL PYRIMIDINES = 2'-FLUORO AND ALL PURINES = 2'-O-ME EXCEPT POSITIONS (N N)
C	SENSE STRAND (SEQ ID NO 695) ALL PYRIMIDINES = 2'-O-ME OR 2'-FLUORO EXCEPT POSITIONS (N N)
	∫ 5'- B-NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
	$3'$ - L- $(N_sN)$ NNNNNNNNNNNNNNNNNNN -5'
	ANTISENSE STRAND (SEQ ID NO 696) ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N)
D	SENSE STRAND (SEQ ID NO 697) ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N) AND ALL PURINES = 2'-DEOXY
	5'- B-NNNNNNNNNNNNNNNNNNNNNNN -3'
	$\int 3'$ - L- $(N_sN)$ NNNNNNNNNNNNNNNNNNN -5'
	ANTISENSE STRAND (SEQ ID NO 694) ALL PYRIMIDINES = 2'-FLUORO AND ALL PURINES = 2'-O-ME EXCEPT POSITIONS (N N)
E	SENSE STRAND (SEQ ID NO 698) ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N)
	5'- B-NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
	3'- L-(N <sub>S</sub> N) NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
	ANTISENSE STRAND (SEQ ID NO 694) ALL PYRIMIDINES = 2'-FLUORO AND ALL PURINES = 2'-O-ME EXCEPT POSITIONS (N N)
	SENSE STRAND (SEQ ID NO 697)
	ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N) AND ALL PURINES = 2'-DEOXY
F	$\int 5'$ - B-NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
	$3'$ - L- $(N_sN)$ NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
	ANTISENSE STRAND (SEQ ID NO 699) ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N) AND ALL PURINES = 2'-DEOXY
	POSITIONS (NN) CAN COMPRISE ANY NUCLEOTIDE, SUCH AS DEOXYNUCLEOTID

ES (eg. THYMIDINE) OR UNIVERSAL BASES

- B = ABASIC, INVERTED ABASIC, INVERTED NUCLEOTIDE OR OTHER TERMINAL CAP THAT IS OPTIONALLY PRESENT
- L = GLYCERYL MOIETY THAT IS OPTIONALLY PRESENT
- S = PHOSPHOROTHIOATE OR PHOSPHORODITHIOATE

# McSwiggen et al. RNA Interference Mediated Treatment of Alzheimer's Disease Using Short Interfering Nucleic Acid (siNA) Serial No. TBA; MBHB02-728-B (400/120) Sheet 5 of 13

# Figure 5

	rig	ure 3	_
		SENSE STRAND (SEQ ID NO 700)	
A	5'-	iB-C A U G G C U G C C A U C U G C G C C T T-iB	-3'
A	<b>3'</b> -	L-T <sub>S</sub> T GUACCGACGGUAGACGCGG	-5'
		ANTISENSE STRAND (SEQ ID NO 701)	
	}		$\downarrow$
	[	SENSE STRAND (SEQ ID NO 702)	
_	5'-	$c \underline{\mathbf{a}} \mathbf{u} \mathbf{g} \mathbf{g} \mathbf{c} \mathbf{u} \mathbf{g} \mathbf{c} \mathbf{c} \underline{\mathbf{a}} \mathbf{u} \mathbf{c} \mathbf{u} \mathbf{g} \mathbf{c} \mathbf{g} \mathbf{c} \mathbf{c} T T$	-3'
B	₹ 3'-	$L-T_STgu\underline{a}ccg\underline{a}cggu\underline{a}g\underline{a}cg\underline{c}gg$	-5'
		ANTISENSE STRAND (SEQ ID NO 703)	
			J
		SENSE STRAND (SEQ ID NO 704)	)
_	5'-	iB-cAuGGcuGccAucuGcGccTT-iB	-3'
C	<b>₹</b> 3'-	L-T <sub>S</sub> TGuAccGAcGGuAGAcGcGG	-5'
		ANTISENSE STRAND (SEQ ID NO 705)	
	Ĺ		لِ
		SENSE STRAND (SEQ ID NO 706)	
n	5'-	iB-cAuGGcuGccAucuGcGccTT-iB	-3'
v	<b>3'-</b>	L-T <sub>S</sub> T gu <u>a</u> c c g <u>a</u> c g g u <u>a</u> g <u>a</u> c g c g g	-5'
		ANTISENSE STRAND (SEQ ID NO 703)	
	}	SENSE STRAND (SEQ ID NO 707)	$\left\{ \right.$
	<u></u>		
E	5'-	iB-cAuGGcuGccAucuGcGccTT-iB	-3'
س	₹ 3'-	$L-T_ST$ gu $\underline{a}$ c c g $\underline{a}$ c g g u $\underline{a}$ g $\underline{a}$ c g c g g	-5' >
		ANTISENSE STRAND (SEQ ID NO 703)	
	}	CENICE CTD AND (SEO ID NO 706)	$\langle$
		SENSE STRAND (SEQ ID NO 706)	
F	5'-	iB-cAuGGcuGccAucuGcGccTT-iB	-3'
Г	₹ 3'-	L-T <sub>S</sub> T G u A c c G A c G G u A G A c G c G G	-5' >
		ANTISENSE STRAND (SEQ ID NO 708)	
	L		J

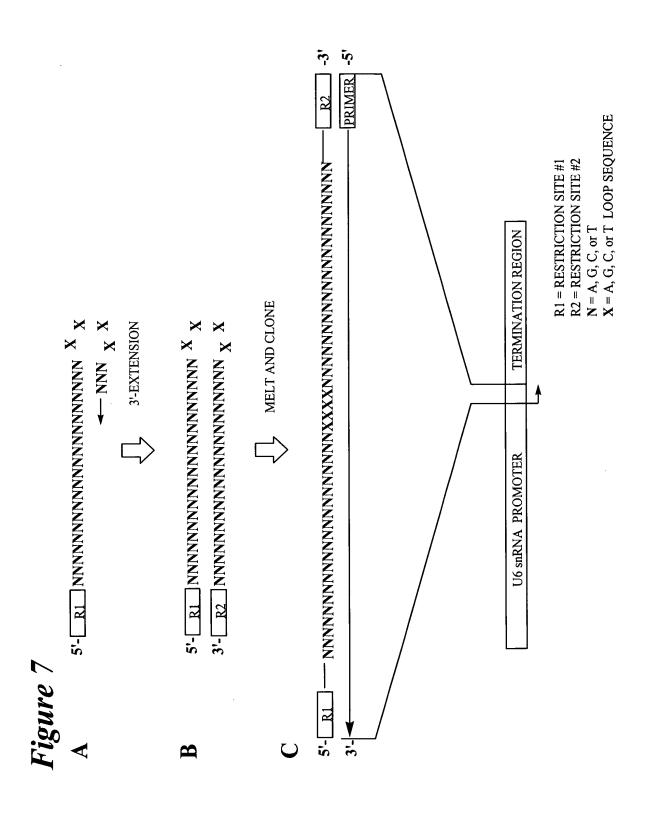
lower case = 2'-O-Methyl or 2'-deoxy-2'-fluoro italic lower case = 2'-deoxy-2'-fluoro underline = 2'-O-methyl

ITALIC UPPER CASE = DEOXY
B = INVERTED DEOXYABASIC
L = GLYCERYL MOIETY OPTIONALLY PRESENT
S = PHOSPHOROTHIOATE OR
PHOSPHORODITHIOATE

# ANTISENSE ANTISENSE ANTISENSE ANTISENSE SENSE SENSE RNAi RNAi Z RNAi ANTISENSE 5' ANTISENSE ANTISENSE **ANTISENSE** SENSE SENSE SENSE n = 0, 1, 2, 3, 47

McSwiggen et al.

RNA Interference Mediated Treatment of Alzheimer's
Disease Using Short Interfering Nucleic Acid (siNA)
Serial No. TBA; MBHB02-728-B (400/120)
Sheet 7 of 13



McSwiggen et al.

RNA Interference Mediated Treatment of Alzheimer's

Disease Using Short Interfering Nucleic Acid (siNA)

Serial No. TBA; MBHB02-728-B (400/120)

Sheet 8 of 13

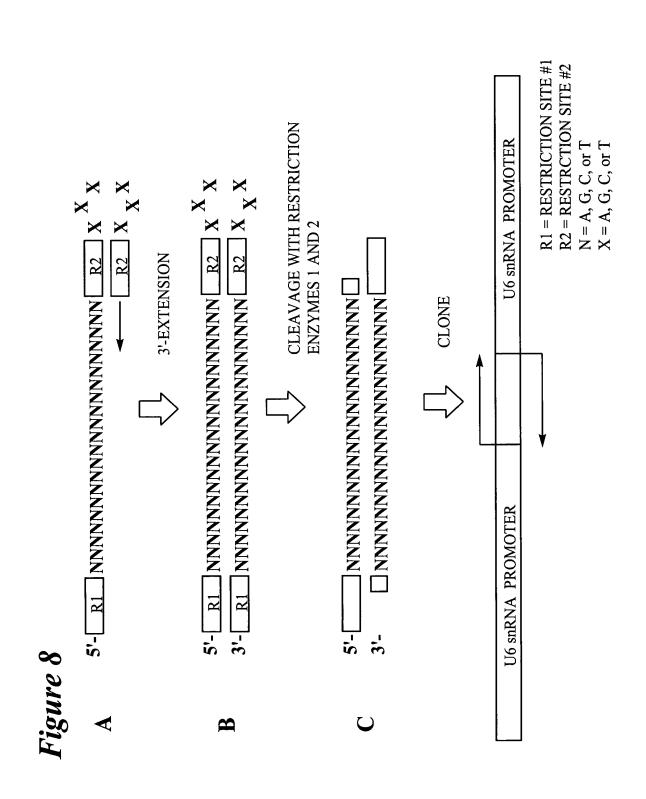
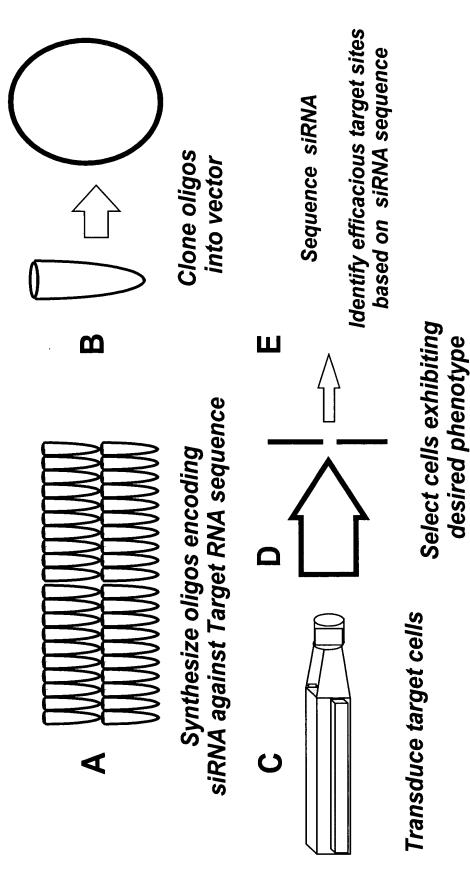
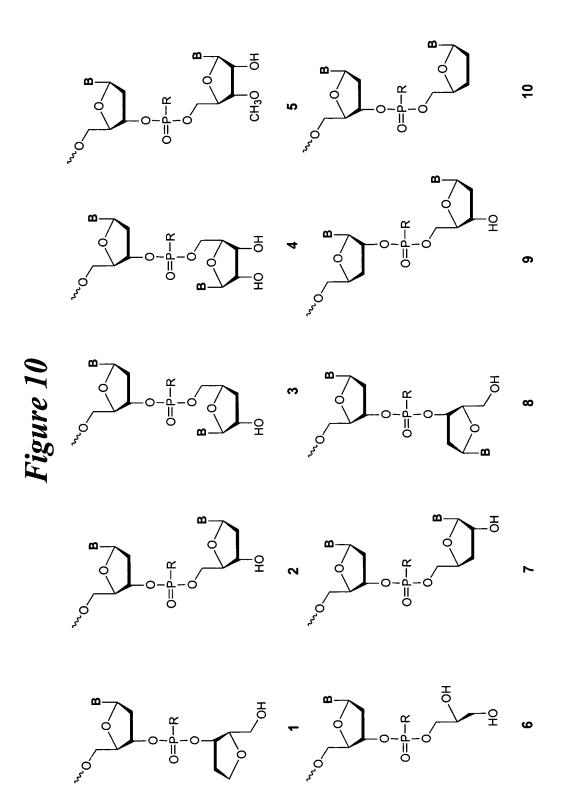


Figure 9: Target site Selection using siRNA





R = O, S, N, alkyl, substituted alkyl, O-alkyl, S-alkyl, alkaryl, or aralkyl
B = Independently any nucleotide base, either naturally occurring or chemically modified, or optionally H (abasic).

# Figure 11: Modification Strategy

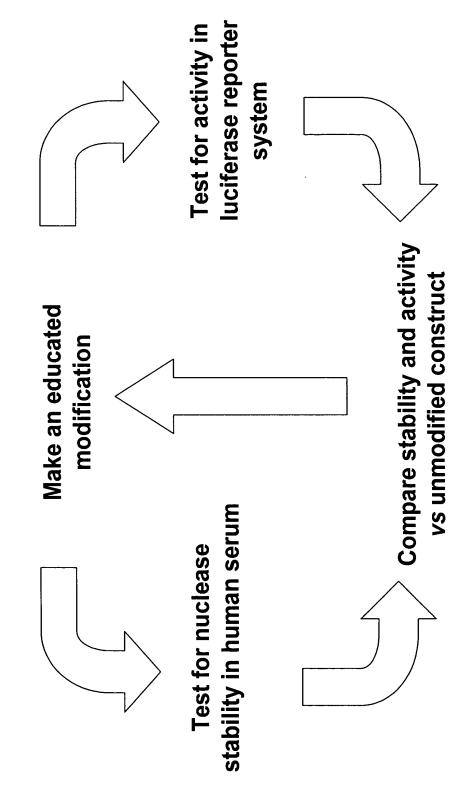


Figure 12: A549 24h BACE mRNA Expression

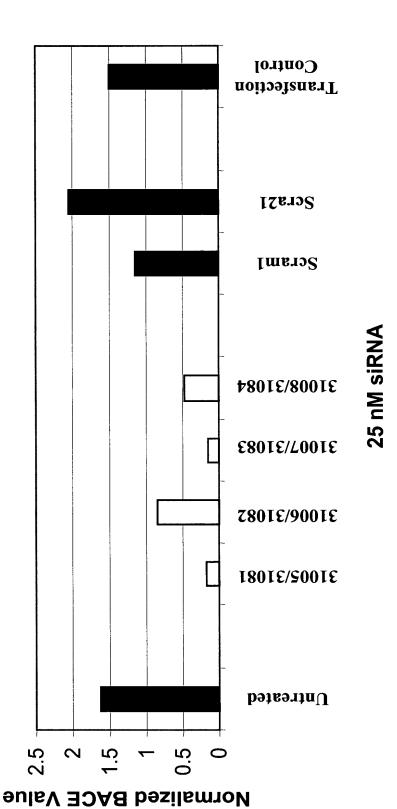
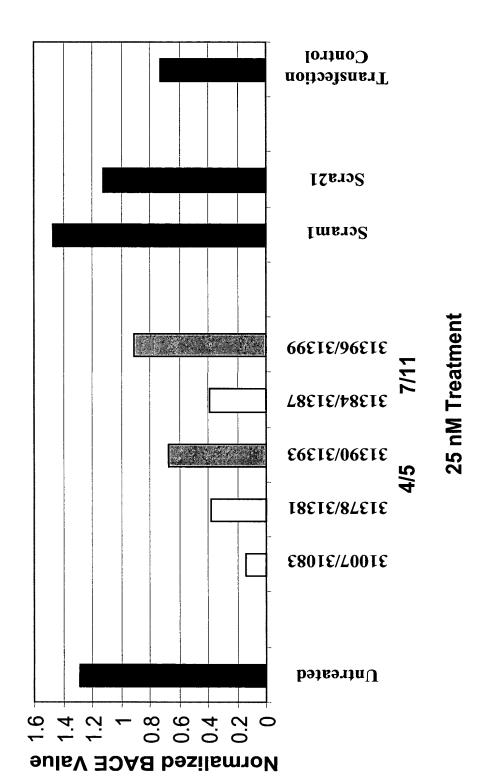


Figure 13: A549 24h BACE mRNA Expression using modified siNA



McSwiggen et al.

RNA Interference Mediated Treatment of Alzheimer's

Disease Using Short Interfering Nucleic Acid (siNA)

Serial No. TBA; MBHB02-728-B (400/120)

Sheet 13 of 13